Office of Environmental Health Hazard Assessment

Joan E. Denton, Ph.D., Director
Headquarters • 1001 I Street • Sacramento, California 95814
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010
Oakland Office • Mailing Address: 1515 Clay Street, 16th Floor • Oakland, California 94612

Terry Tamminen
Agency Secretary

Arnold Schwarzenegger
Governor

August 11, 2004

AUG 16 2004

Dr. C. W. Jameson
National Toxicology Program,
Report on Carcinogens
79 Alexander Drive
Building 4401, Room 3118
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Re: Petition to Delist DEHP from NTP Report on Carcinogens

Dear Dr. Jameson:

Thank you for providing the opportunity to comment on the important subject of the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP). The attached comments are responsive to a petition to delist DEHP from the National Toxicology Program Report on Carcinogens, Twelfth Edition. Attached you will find detailed comments in many of the areas that I consider critical in establishing whether DEHP should or should not be removed from the list of chemicals that are "reasonably anticipated to be human carcinogens." Copies of the most relevant supporting studies are also included as part of this submission.

If you would like to discuss these issues further, please contact me at (510) 622-3185, or at the Oakland Office mailing address above.

Sincerely,

John B. Faust, Ph.D.
Staff Toxicologist
Reproductive and Cancer Hazard
Assessment Section

Enclosures

Considerations Regarding the Carcinogenicity of Di(2-Ethylhexyl)Phthalate

John B. Faust, Ph.D.

The determination as to whether di(2-ethylhexyl)phthalate (DEHP) should remain on NTP's list of chemicals "reasonably anticipated to be human carcinogens" hinges on assessing a large body of data investigating whether the tumors caused by DEHP should not be considered relevant in human cancer risk assessment.

It is reasonable and appropriate to assume that chemicals that cause cancer in experimental animals will also do so in humans. The weight of evidence to overcome this assumption must be high because the consequences of error may be severe.

The presumption that chemicals that cause cancer in experimental animals will also do so in humans – a presumption formalized in carcinogen risk assessment guidelines – presents a heavy burden in the assessment of mechanistic evidence purporting to challenge the relevance of the animal tumor findings to humans. The presumption of relevance is sound, one codified by the National Toxicology Program (NTP), the International Agency for Research on Cancer (IARC), and the U.S. Environmental Protection Agency (U.S. EPA). The following is taken from the Introduction to the Tenth Edition of the NTP Report on Carcinogens:

> "It is not possible to predict with complete certainty, from animal studies alone, which substances will be carcinogenic in humans; however, generally known human carcinogens that have been tested adequately in laboratory animals also produce cancers in laboratory animals. In many cases, a substance was first found to cause cancer in animals and only later was confirmed to cause cancer in humans (Huff 1993). Experimental cancer research is based on the scientific assumption that substances causing cancer in animals will have similar effects in humans. How laboratory animals respond to substances (including cancer and other illnesses) does not always strictly correspond to how people will respond; however, laboratory animal studies remain the best tool for detecting potential human health hazards of all kinds, including cancer (OTA 1981, Tomatis *et al.* 1997)."

As you are aware, the NTP Report on Carcinogens cites the following listing criteria for making determinations about carcinogenesis:

> "Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are **compelling** data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans." [Emphasis added]

A primary reason for the presumption of relevance is that the consequences of error may be severe. Removal of a known animal carcinogen from a list of reasonably anticipated carcinogens to humans may result in changes in how such a chemical is regulated. Human exposures may become more common and warnings of exposures may cease. For this reason, the mechanistic evidence used to support a determination that a known animal carcinogen does not also cause cancer in humans must be extensive, rigorous, and compelling.

Acceptance that the mode/mechanism of action of carcinogenicity of di(2-ethylhexyl)phthalate (DEHP) is not relevant to humans requires consideration of the entirety and adequacy of the studies of human tissues and cell systems. A few notable points:

Considerations Specific to DEHP

Observations

- DEHP, a peroxisome proliferator activated receptor-alpha (PPAR-alpha) agonist, has been shown to cause tumors in rats and mice, with evidence for the development of liver adenomas and carcinomas, pancreatic adenomas, and mononuclear cell leukemia (NTP, 1982; David *et al.,* 2000; Rao *et al.*, 1990). Studies with a number of other PPAR-alpha agonists have shown induction of a triad of tumors: Liver, pancreas, and Leydig cell tumors (Biegel *et al.*, 2001; Obourn *et al.*, 1997). DEHP produced Leydig cell hyperplasia in rats (Akingbemi et al., 2004).
- DEHP and other PPAR-alpha agonists have not been adequately tested for carcinogenicity in species other than rats and mice, although there has been limited testing in other species. Of the studies that approach life-term duration, dosing in studies of hamsters was inadequate (DEHP: Schmezer *et al.*, 1988) and studies in marmosets were less than lifetime, which can be as long as 15 years for this species (clofibrate: Tucker and Orton, 1993; DEHP: Kurata *et al.*, 1998; ciprofibrate: Graham *et al.*, 1994), so none could rule out a carcinogenic effect in each respective species.
- There are no epidemiologic studies of DEHP *per se* that adequately assess whether DEHP increases the risk of cancer in humans. Furthermore, clinical trials of the PPAR-alpha agonist pharmaceuticals clofibrate and gemfibrozil have not been conducted for adequate duration to rule out carcinogenic effects.

Data Gaps and Considerations

- DEHP has been shown to induce benign pancreatic adenomas in rats (NTP, 1982; David *et al.,* 2000; Rao *et al.*, 1990). Other PPAR-alpha agonists have induced malignant pancreatic tumors (Obourn, 1997). The mechanism by which these pancreatic effects occur is unknown, but does not involve peroxisome proliferation.
    - ➢ How much weight should the effects of DEHP on pancreatic tumor development be afforded, particularly in light of the common finding with other PPAR-alpha agonists?

- Several potential mechanisms/modes of action have been investigated since DEHP was first identified as a carcinogen by NTP in 1982. These include, but are not limited to, stimulation of oxidative stress, DNA synthesis, and suppression of apoptosis.
  - ➢ Is the experimental evidence regarding the potential mode(s) of carcinogenic action for DEHP sufficiently compelling to rule out with confidence the possibility that other mechanisms/modes are involved?
  - ➢ Is it necessary to establish a mode of action to make a determination about relevance to humans?
- Recent data have suggested that DNA damage may result from exposure of both rodent and human cells to PPAR-alpha agonists including MEHP, a primary and active metabolite of DEHP (Deutsch *et al.*, 2001; Anderson *et al.*, 1999; Kleinsasser *et al.*, 2004a; Kleinsasser *et al.*, 2004b).
  - ➢ Do these data have weight with respect to known or unknown modes of action? Are they a significant cause for concern with respect to establishing relevance of animal findings to humans?
- The duration of the bioassay in the PPAR-alpha null mice for DEHP was 24 weeks (Ward *et al.*, 1998) and 11 months for the PPAR-alpha agonist Wy-14643 (Peters *et al.*, 1997).
  - ➢ Are these studies of adequate duration to rule out any carcinogenic effects in this strain of mice?
- Hasmall *et al.* (2000) and Parzefall *et al.* (2001) presented a series of studies demonstrating that the *in vitro* DNA synthesis response of hepatocytes to PPAR-alpha agonists (one of the proposed modes of action) requires the presence of at least two cell types, parenchymal hepatocytes and non-parenchymal cells (possibly Kupffer cells). The published studies of human hepatocytes – a key set of studies generally relied on to make conclusions regarding human responsiveness – have not identified whether multiple cell types, particularly Kupffer cells, were present under the conditions of the assays. Significant differences between how human and rat hepatocytes are prepared may influence the relative proportion of parenchymal hepatocytes and non-parenchymal cells.
  - ➢ If the DNA synthesis hypothesis as a mechanism is considered viable for DEHP and other PPAR-alpha agonists and if the Hasmall and Parzefall data are considered reliable, has there been adequate validation of the *in vitro* studies of human liver cells demonstrating they are essentially equivalent to those performed with rat liver cells?
- Humans are clearly responsive to PPAR-alpha agonists (*e.g.*, the hypolipidemic pharmaceuticals clofibrate and gemfibrozil). As a likely receptor-mediated phenomenon and given the nature of the PPAR-alpha receptor (a nuclear receptor regulating gene expression), there is the potential for different species to be responsive to PPAR-alpha agonists on a gene-by-gene basis through gene-specific response elements (PPREs) in the DNA sequences proximal to regulated genes.
  - ➢ How convincing is the evidence that protein enzymes such as acyl-CoA oxidase or CYP4A are appropriate indicators of sensitivity to the carcinogenic effects of DEHP

or PPAR-alpha agonists? Is it appropriate to extend these "markers" of sensitivity to other species with respect to carcinogenicity?

- How important is it to identify genes/proteins, or battery of genes/proteins, that are on the "pathway" to carcinogenesis before assessing potential sensitivity in a given species and test system?

- Gene transfection studies in the human cell line HepG2, derived from a human hepatoblastoma, have suggested differences in responsiveness of individual genes, likely based upon differences in individual response elements (PPREs) (Hsu *et al.*, 2001; Lawrence *et al.*, 2001).
  - Is the broad difference in responsiveness of rodents (*e.g.*, peroxisomal fatty acid oxidation) and humans (*e.g.*, mitochondrial fatty acid oxidation) observed thus far sufficient to conclude that humans are different from rodents with respect to the as yet unidentified genes or battery of genes related to *carcinogenesis* as well as those related to fatty acid metabolism and homeostasis?
- Liver is the common site of DEHP-induced tumor development in rats and mice. The mode of action is unknown, other than a dependence on the PPAR-alpha receptor, with receptor-mediated stimulation of cell proliferation, suppression of apoptosis, and oxidative stress having been proposed and studied as potential modes of action.
  - Should potential tumor site-concordance for PPAR-alpha agonists between experimental animals and humans be assumed? Is it reasonable to assume that other tissues or organs may be at risk in humans?
  - What is the confidence level in the data examining PPAR-alpha receptor status in humans, that is, in which tissues has PPAR-alpha status been assessed in humans?
  - Does establishing the susceptibility of human tissues other than the liver to agonistic actions via PPAR-alpha require further investigation? [There is limited evidence in this regard, but a systematic evaluation has not been conducted.]
  - Should the studies in rodents regarding PPAR-alpha receptor status's vulnerability to change (several-fold diurnal fluctuations and susceptibility to modulation by glucocorticoids characterized by Lemberger *et al.*, 1994 and 1996) be taken into consideration for humans?

References:

Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP (2004). Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. *Proc Natl Acad Sci U S A* **101**(3):775-80.

Anderson D, Yu TW, Hincal F (1999). Effect of some phthalate esters in human cells in the comet assay. *Teratog Carcinog Mutagen* **19**(4):275-80.

Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC (2001). Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci* **60**(1):44-55.

David RM, Moore MR, Finney DC, Guest D (2000). Chronic toxicity of di(2-ethylhexyl)phthalate in rats. *Toxicol Sci* **55**(2):433-43.

Deutsch WA, Kukreja A, Shane B, Hegde V (2001). Phenobarbital, oxazepam and Wyeth 14,643 cause DNA damage as measured by the Comet assay. *Mutagenesis* **16**(5):439-42.

Graham MJ, Wilson SA, Winham MA, Spencer AJ, Rees JA, Old SL *et al.* (1994). Lack of peroxisome proliferation in marmoset liver following treatment with ciprofibrate for 3 years. *Fundam Appl Toxicol* **22**(1):58-64.

Hasmall SC, West DA, Olsen K, Roberts RA (2000). Role of hepatic non-parenchymal cells in the response of rat hepatocytes to the peroxisome proliferator nafenopin in vitro. *Carcinogenesis* **21**(12):2159-65.

Hsu MH, Savas U, Griffin KJ, Johnson EF (2001). Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor α in HepG2 cells. *J Biol Chem* **276**(30):27950-8.

Kleinsasser NH, Harreus UA, Kastenbauer ER, Wallner BC, Sassen AW, Staudenmaier R *et al.* (2004a). Mono(2-ethylhexyl)phthalate exhibits genotoxic effects in human lymphocytes and mucosal cells of the upper aerodigestive tract in the comet assay. *Toxicol Lett* **148**(1-2):83-90.

Kleinsasser NH, Juchhoff J, Wallner BC, Bergner A, Harreus UA, Gamarra F *et al.* (2004). The use of mini-organ cultures of human upper aerodigestive tract epithelia in ecogenotoxicology. *Mutat Res* **561**(1-2):63-73.

Kurata Y, Kidachi F, Yokoyama M, Toyota N, Tsuchitani M, Katoh M (1998). Subchronic toxicity of di(2-ethylhexyl)phthalate in common marmosets: lack of hepatic peroxisome proliferation, testicular atrophy, or pancreatic acinar cell hyperplasia. *Toxicol Sci* **42**(1):49-56.

Lawrence JW, Li Y, Chen S, DeLuca JG, Berger JP, Umbenhauer DR *et al.* (2001). Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) alpha. PPARalpha fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expression. *J Biol Chem* **276**(34):31521-7.

Lemberger T, Staels B, Saladin R, Desvergne B, Auwerx J, Wahli W (1994). Regulation of the peroxisome proliferator-activated receptor α gene by glucocorticoids. *J Biol Chem* **269**(40):24527-30.

Lemberger T, Saladin R, Vazquez M, Assimacopoulos F, Staels B, Desvergne B *et al.* (1996). Expression of the peroxisome proliferator-activated receptor α gene is stimulated by stress and follows a diurnal rhythm. *J Biol Chem* **271**(3):1764-9.

NTP (1982). National Toxicology Program. Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F1 Mice (Feed Study). NTP-80-37, Technical Report Series No. 217.

National Toxicology Program (2002). Report on Carcinogens, Tenth Edition; U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, December 2002.

Obourn JD, Frame SR, Bell RH Jr, Longnecker DS, Elliott GS, Cook JC (1997). Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferator Wyeth-14,643. *Toxicol Appl Pharmacol* **145**(2):425-36.

Parzefall W, Berger W, Kainzbauer E, Teufelhofer O, Schulte-Hermann R, Thurman RG (2001). Peroxisome proliferators do not increase DNA synthesis in purified rat hepatocytes. *Carcinogenesis* **22**(3):519-23.

Peters JM, Cattley RC, Gonzalez FJ (1997). Role of PPAR-alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator Wy-14,643. *Carcinogenesis* **18**(11):2029-33.

Rao MS, Yeldandi AV, Subbarao V (1990). Quantitative analysis of hepatocellular lesions induced by di(2-ethylhexyl)phthalate in F-344 rats. *J Toxicol Environ Health* **30**(2):85-9.

Schmezer P, Pool BL, Klein RG, Komitowski D, Schmähl D (1988). Various short-term assays and two long-term studies with the plasticizer di(2-ethylhexyl)phthalate in the Syrian golden hamster. *Carcinogenesis* **9**(1):37-43.

Tucker MJ, Orton TC (1993). Toxicological studies in primates with three fibrates. In: Gibson G, Lake B, eds. *Peroxisomes Biology and Importance in Toxicology Medicine*. London: Taylor & Francis, pp. 425-63.

Ward JM, Peters JM, Perella CM, Gonzalez FJ (1998). Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor α-null mice. *Toxicol Pathol* **26**(2):240-6.